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Tandem Duplication of the *MLL* Gene in Myelodysplastic Syndrome-Derived Overt Leukemia With Trisomy 11

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Trisomy 11 as a sole chromosomal abnormality is a rare aberration observed in myelodysplastic syndrome (MDS) or acute myeloblastic leukemia (AML). Recently a partial tandem duplication of the *MLL* gene, located on chromosome band 11q23, has been identified in de novo AML with trisomy 11. We describe a 72-year-old woman suffering from MDS-derived overt leukemia with trisomy 11 and a tandem duplication of the *MLL* gene. At first the patient was found to have myeloblasts with Auer rods in the peripheral blood and diagnosed as MDS, refractory anemia with excess of blasts in transformation (RAEB-T). After 2 months a picture of overt leukemia (AML; M2) developed as shown by an increased number of myeloblasts. Various chemotherapy regimens had little effect, and she died of disease progression 15 months after admission. During her clinical course, the chromosome analyses consistently showed 47,XX, +11. Southern blot analysis of leukemic blasts on admission and in accelerated phase revealed identical rearranged bands of the *MLL* gene. Fluorescence in situ hybridization analysis excluded the possibility of masked translocation of the *MLL* gene to other chromosomes. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis using a forward exon 6 primer and a backward exon 3 primer demonstrated an in-frame fusion of exon 8 with exon 2. Our results indicated that a partial tandem duplication of exons 2–8 of the *MLL* gene could be observed in MDS-derived overt leukemia as well as de novo AML with trisomy 11. Am. J. Hematol. 55:41–45, 1997. © 1997 Wiley-Liss, Inc.

Key words: tandem duplication; *MLL* gene; trisomy 11; acute myeloblastic leukemia; myelodysplastic syndrome

INTRODUCTION

Trisomy 11 as a sole abnormality is an infrequent nonrandom chromosomal aberration observed in myelodysplastic syndrome (MDS) or acute myeloblastic leukemia (AML). More than 50 cases have been reported, but MDS/AML with trisomy 11 does not correlate with any specific subtype in the French–American–British (FAB) classification [1–6]. AML with trisomy 11 was shown to be associated with a stem/progenitor cell immunophenotype with myeloid antigen expression and characterized by poor response to standard chemotherapy and an unfavorable prognosis [6]. Recently, molecular characterization of de novo AML with trisomy 11 has revealed that the *MLL* gene (also called *ALL-1, HRX*, or *Htrx*) was rearranged in spite of lacking cytogenetic evidence of 11q23 translocations and that a partial tandem duplication of the *MLL* gene was identified [7–10]. The duplicated region of the *MLL* gene spanned mainly exons 2–6 or exons 2–8 [8–10]. The resultant ''self-fusion'' of proto-oncogene represents a new genetic mechanism for leukemogenesis. We report the first case of MDS-derived overt leukemia with trisomy 11 and a tandem duplication of the *MLL* gene.

CASE REPORT

A 72-year-old woman was found to have 4% myeloblasts with Auer rods in the peripheral blood, although

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the blood cell count was within the normal range, i.e., white blood cell (WBC) 4.9×10^9/L, hemoglobin (Hb) 12.2 g/dl, and platelet 320×10^9/L in July 1994. Bone marrow examination revealed 8.2% myeloblasts with Auer rods and marked dysplasia of neutrophils and megakaryocytes. She was diagnosed as having MDS, refractory anemia with excess of blasts in transformation (RAEB-T), according to the FAB classification. The karyotype of bone marrow cells was 47,XX, +11 in all 20 metaphases analyzed. In September 1994, she was admitted to our hospital because of increase of myeloblasts. Peripheral blood showed WBC 20×10^9/L with 40% myeloblasts, Hb 8.1 g/dl, and platelet 100×10^9/L. Bone marrow was hypercellular with 62% myeloblasts, which were strongly positive for myeloperoxidase and chroloacetate esterase, but negative for α-napthyl butyrate esterase and periodic acid–Schiff (PAS) stainings. In surface marker analysis, myeloblasts were 26% positive for CD13, 89% for CD33, 52% for CD34, and 79% for HLA-DR. Chromosome analysis of bone marrow cells showed 47,XX, +11 [8]/48,XX, +11, +13 [1]. She was diagnosed as having MDS-derived AML M2, according to the FAB classification.

She was treated with cytarabine ocfosfate, 300 mg/day, for 3 weeks, without apparent effect. Low-dose administration of etoposide and aclarubicin was then repeated. Nevertheless, leukemic blasts increased rapidly since June 1995. She died of progressive disease and pneumonia in November 1995, despite administration of intermediate-dose cytosine arabinoside on several occasions.

Peripheral blood cells were obtained from the patient with informed consent on August 29, 1995. At this time, peripheral blood showed WBC 25×10^9/L (81% myeloblasts, 4% neutrophils, 14% lymphocytes, and 1% monocytes), Hb 8.3 g/dl and platelet 17×10^9/L. They were used for the following studies. In addition, bone marrow cells were obtained on admission (September 28, 1994) and used for Southern blot analysis.

MATERIALS AND METHODS

Chromosome Analysis

Chromosome studies were performed on short-term culture of the cells from peripheral blood by the Giemsa banding technique. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN).

Southern Blot Analysis

Mononuclear cells were separated from bone marrow cells or peripheral blood cells of the patient on Ficoll-Metrizoate (Lymphoprep, NYCOMED PHARMA AS, Oslo, Norway). High-molecular-weight DNAs were extracted from these cells and human placenta by proteinase K digestion followed by phenol–chloroform extraction and ethanol precipitation. Five μg of DNA was digested with *Bam*HI or *Hin*dIII, separated by electrophoresis on 0.8% agarose gel, transferred to nylon filters, and hybridized with the Photobiotin (Life Technologies, Gaithersburg, MD)-labeled probe x. Probe x (kindly provided by Dr. M. Seto, Aichi Cancer Center, Nagoya, Japan) is the 0.9-kb *Bam*HI cDNA fragment of the *MLL* gene containing exons 5–11 [11].

Fluorescence In Situ Hybridization Analysis

The probes used in the fluorescence in situ hybridization (FISH) assay were the yeast artificial chromosome (YAC) clone 13HH4 mapped to 11q23 (kindly provided by Dr. Lyndal Kearney, MRC Molecular Haematology Unit, Institute of Molecular Medicine, Oxford, UK) and the YAC clone 806E1 mapped to 11q24 (kindly provided by Dr. Licia Selleri, Department of Pathology, Stanford University School of Medicine, CA). These probes were labeled by standard nick-translation using biotin-11-dUTP (Sigma, St. Louis, MO) or digoxigenin-11-dUTP (Boehringer, Mannheim, Germany), respectively. Two-color FISH was carried out as described elsewhere [12].

Reverse Transcriptase-Polymerase Chain Reaction Analysis

Total RNA was extracted from mononuclear cells of the patient and normal control by standard guanidium thiocyanate/phenol/chloroform method using RNAzol™B (Biotecx Lab., Houston, TX). First-strand DNA was synthesized from 1 μg of total RNA in 30 μl of the reaction mixture containing 50 mM Tris–HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 500 μM dNTPs, 20 units of RNAsin, 100 pmol of RT-primer, and 20 units of M-MLV reverse transcriptase (Life Technologies). The mixture was incubated at 37°C for 1 hr. PCR was carried out in the reaction mixture by adding 5 μl of 10× PCR buffer (100 mM Tris–HCl pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin), 200 μM dNTPs, 2.5 units of Taq DNA polymerase (Promega, Madison, Wisconsin) and 50 pmol of each oligonucleotide described below. After an initial denaturation at 94°C for 1 min, 35 cycles of PCR (denaturation at 95°C for 60 sec, annealing at 65°C for 60 sec, and extension at 72°C for 90 sec) were performed in a thermal cycler (Astec PC800, Astec, Fukuoka, Japan) followed by a final round of extension at 72°C for 10 min. Five μl of the PCR products was run on a 2% NuSieve/1% agarose gel and stained with ethidium bromide. The DNA of the ϕX174 digested by *Hae*III was used for a size marker.

The sequences of PCR primers were designed as follows: 6.1 (5′-GTC CAG AGC AGA GCA AAC AG-3′) from exon 6 and 3.2c (5′-ACA CAG ATG GAT CTG AGA GG-3′) from exon 3 [8].

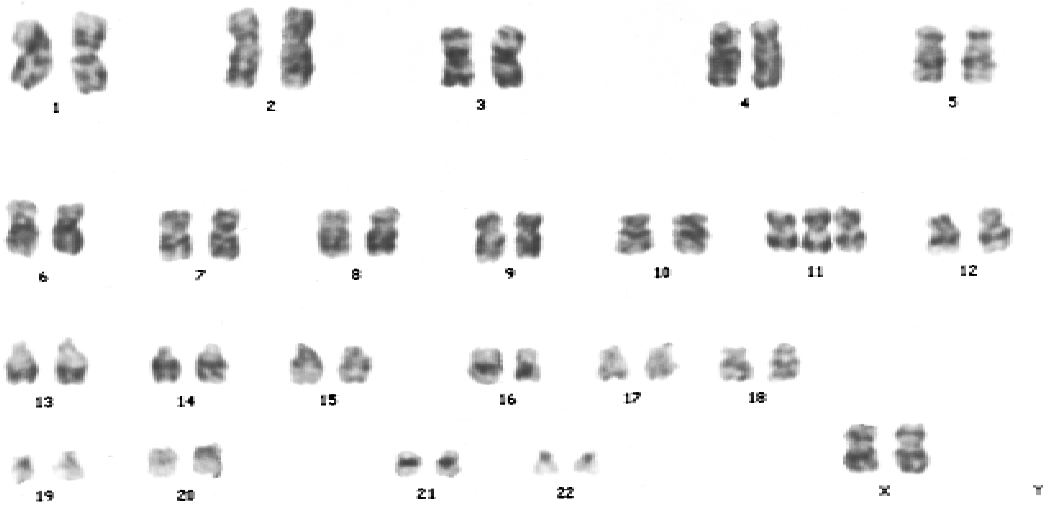

Fig. 1. Karyotype of the leukemic blasts by the Giemsa banding technique: 47,XX, +11.

DNA Sequence Analysis

DNA sequencing of the amplified RT-PCR products purified from agarose gel was carried out directly, using Taq Dye Deoxy™ Terminator Cycle Sequencing kit (Perkin Elmer, Foster City, CA) and DNA sequencing system (ABI model 373A; Perkin Elmer) according to the manufacturer's instructions.

RESULTS

Chromosome Analysis

The karyotypes of 20 analyzed metaphases of the peripheral blood cells were determined as follows: 47,XX, +11 [17]/47,X, Xq+, +11 [2]/45,XX, −4, −9, +11 [1] (Fig. 1).

Southern Blot Analysis

Southern blot analysis was performed for detection of the *MLL* gene rearrangement. DNA from the peripheral blood cells of the patient in accelerated phase (August 29, 1995) was digested with *Bam*HI or *Hin*dIII and hybridized with the *MLL* cDNA probe containing exons 5–11. Abnormal rearranged bands were observed in addition to germline bands in both digestions (Fig. 2, lane 2). Furthermore, we examined *MLL* gene rearrangement using DNA sample from the bone marrow cells on admission (September 28, 1994). Faint rearranged bands of the same size were identified (Fig. 2, lane 1).

FISH Analysis

To exclude the possibility of masked translocation with chromosome 11 and other chromosomes, we carried out FISH analysis. YAC clones 13HH4 at 11q23 containing the *MLL* gene and 806E1 at 11q24 were used as a probe. Both signals were identified on the correct region of chromosome 11 and they were not splitted (data not shown).

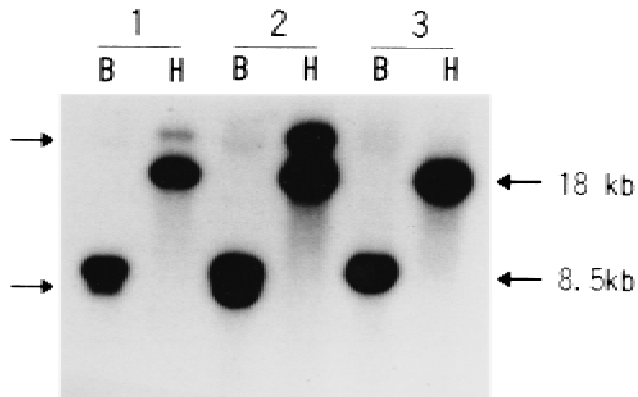

Fig. 2. Southern blot analysis of the *MLL* gene. DNAs were digested with *Bam*HI or *Hin*dIII, separated by electrophoresis on 0.8% agarose gel, and transferred to the nylon filter. The filter was hybridized with the *MLL* cDNA probe containing exons 5–11. (1) leukemic blasts of the patient on admission (September 28, 1994); (2) leukemic blasts in accelerated phase (August 29, 1995); (3) human placenta. B: *Bam*HI, H: *Hin*dIII. *Arrows* (right side), 8.5-kb and 18-kb germline bands; *arrows* (left side), rearranged bands.

RT-PCR and Nucleotide Sequence Analysis

For characterization of a predicted tandem partial duplication of the *MLL* gene, a forward primer on exon 6 and a backward primer on exon 3 were designed, and RT-PCR was performed. One major band and one minor band were detected only in the lane of the patient (Fig. 3). Sequence analysis of two PCR products showed an in-frame fusion of exon 8 with exon 2 (474 bp) and an in-frame fusion of exon 7 with exon 2 (360 bp) (data not

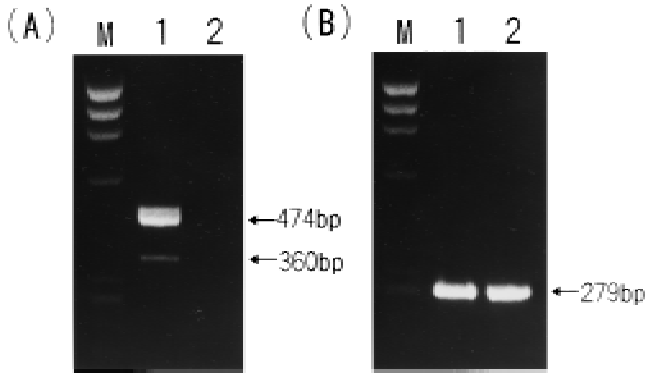

Fig. 3. RT-PCR analysis of the partially duplicated *MLL* transcript. A: RT-PCR products using an exon 6 forward primer and an exon 3 backward primer were observed only in the leukemic blasts of the patient (*lane 1*). *Arrows,* a major band (474 bp) and a minor band (360 bp). Negative control (*lane 2;* normal mononuclear cells) did not yield visible PCR product. B: β-actin cDNA was also amplified as a control.

shown). These results indicated a tandem duplication of exons 2–8 and an alternative splicing of exon 8 as previously reported [9,10,14,15].

DISCUSSION

This report identifies a partial tandem duplication of the *MLL* gene in a 72-year-old woman suffering from MDS-derived overt AML with trisomy 11. There was a possibility that the diagnosis of the patient might be de novo AML with trilineage myelodysplasia, in view of rapid progression from MDS to AML within 2 months. However, it is very difficult to differentiate clearly MDS-derived overt AML from de novo AML with myelodysplasia. In the present case, we made the diagnosis of MDS at initial examination because myeloblasts in peripheral blood and bone marrow were only 4% and 8.2%, respectively.

Fourteen cases of AML with trisomy 11 as a sole abnormality and *MLL* gene rearrangement have been reported to date. Many of them occurred in AML M1 or M2 in the FAB classification, while AML with 11q23 translocations are usually associated with M4 or M5 subtypes. A median age was relatively high. At first, Caligiuri et al. [7] detected *MLL* gene rearrangements in three of four patients with de novo AML and trisomy 11. Slovak et al. [6] reported that *MLL* gene rearrangements were observed in two of four AML patients characterized by trisomy 11. Myelodysplastic features of these two patients were not assessed. Bernard et al. [10] showed *MLL* gene rearrangements in two of three patients with trisomy 11 AML. One patient without *MLL* rearrangement was AML M2 following refractory anemia with excess of blasts, but two patients with rearrangements were AML M4 and M1. Recently, Caligiuri and colleagues also showed that the *MLL* gene was rearranged in 10 of 11 patients (including four patients [7]) of AML with trisomy 11 as a sole abnormality [9]. Therefore, rearrangements of the *MLL* gene were found with high incidence (79%) in AML with trisomy 11 because 15 of 19 patients totally tested were positive. Early evaluation of *MLL* gene rearrangements in AML with trisomy 11 would be useful for selection of the appropriate risk-directed therapy as *MLL* rearrangements are usually associated with a poor prognosis [13]. Furthermore, there was no apparent evidence of previous history of myelodysplasia in these cases, except for the one case. This patient reported by Caligiuri et al. (UPN 137.1) had a 3-month history of MDS. *MLL* gene rearrangement was shown by Southern blot analysis. However, RT-PCR was not done because no material was available [9]. Our results, more important, apparently indicated that tandem duplication of the *MLL* gene could be observed in MDS-derived overt AML as well as de novo AML with trisomy 11.

On the other hand, *MLL* gene rearrangements have never been identified in MDS with trisomy 11, although trisomy 11 is widely observed in MDS, de novo AML, and overt AML [1–6]. Caligiuri et al. examined only one patient with MDS (RAEB-T) and trisomy 11 (UPN 110), and *MLL* gene rearrangement was detected by neither Southern blot nor RT-PCR analysis [9]. The karyotype in this patient was not trisomy 11 alone but accompanied by additional abnormalities, i.e., 45,XY, der(5;17)(p10;q10), +11, −19, add(20)(q13.3). It is suggested that the molecular mechanism of leukemogenesis in this patient may be different from tandem duplication of the *MLL* gene, although it can be found in some cases of AML with trisomy 11 or +11q accompanied by other cytogenetic abnormalities. In the present case, the karyotype already showed trisomy 11 when MDS was diagnosed, but unfortunately we could not examine *MLL* rearrangement. We detected *MLL* gene rearrangement by Southern blot analysis when she was admitted and diagnosed as having AML. The sizes of rearranged bands on admission were same as those in the accelerated phase. Therefore, tandem duplication of the *MLL* gene had supposedly occurred on admission, although RT-PCR was not performed at this time. This result clearly indicated that *MLL* gene rearrangement was not a genetic event induced by chemotherapy including etoposide but a molecular defect occurred in the early phase of the disease. It is also suggested that *MLL* gene rearrangement might have been acquired during disease progression from MDS to AML.

In the literature, four cases showed a fusion of exon 6 with exon 2 and four cases, including the present case, showed a fusion of exon 8 with exon 2 [8–10]. Tandem duplications of exon 2 to exon 7, exon 2 to exon 9, and exon 4a to exon 6 were also reported [9]. Therefore, duplication endpoints are within the breakpoint cluster

region (exons 5–11) in 11q23 translocations. In any case, the encoded MLL proteins had duplicated N-terminal domains containing the AT hook and DNA methyltransferase motifs. The resultant dissociation of MLL N-terminal domains from regulatory C-terminal regions is proposed to be critical for leukemic transformation with trisomy 11 [13]. These N-terminal regions are also predicted to participate in the chimeric proteins that result from translocations involving the *MLL* gene with other chromosomes.

MLL gene rearrangements were also detected in 2 of 19 de novo AML patients with normal karyotype, one of which was the result from a fusion of exon 8 with exon 2 [7,14]. Recently Yu et al. [15] reported that 7 of 34 de novo AML patients with normal karyotype had a tandem partial duplication of exon 2 to 6 or exon 2 to 8 of the *MLL* gene. In addition, three patients showed six, seven, and eight cDNA isoforms, although these multiple RNA splicings of the *MLL* gene have never been detected in AML with trisomy 11. However, except for these splicings, the duplicated region of the gene seems to be indistinguishable between AML with trisomy 11 and normal karyotype. With regard to genomic fusion points, *Alu*-mediated homologous recombinations were observed in both cases [10,14]. Accumulation of more cases would be required to elucidate the difference of molecular mechanism between trisomy 11 and normal karyotype and functional significance of a partially duplicated *MLL* gene for leukemogenesis.

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